Acridine Orange

Catalog #6130, 0.5 mL at 1 mM (266 µg/mL)

FOR RESEARCH USE ONLY.
Not for use in diagnostic procedures.

Stain lysosomes and nuclei in cultured cells.

INTRODUCTION

Acridine orange (AO, catalog #6130) is an orange/red fluorescent chelating dye used to reveal lysosomes and nuclei in cultured cells. Due to its metachromatic properties, AO is commonly used in fluorescence microscopy and flow cytometry analysis of cellular physiology and cell cycle status[1, 2].

AO is a slightly cationic, lipophilic, weak base capable of permeating cell and organelle membrane structure[3]. Although quite cell permeant in the neutral form, once protonated, this dye tends to become trapped on the low pH side of the membrane barrier leading to accumulation in acidic organelle structures[1, 2, 4-6]. Proton pump driven lysosomal acidity generates a significant pH gradient resulting in the efficient concentration of AO within the lysosome organelles[6]. This is sufficient to create intra-lysosomal concentrations leading to precipitation of AO into aggregated granules. These oligomeric structures exhibit a red shift (640 nm) compared to the monomeric AO that emits at 525 nm[7].

As AO exhibits a very broad emission range, one of several filter pairings on the fluorescence microscope can be used to view this stain. Using an excitation filter of 550 nm (540-560 nm) with a long pass >610 nm emission/barrier filter, lysosomes appear red. When illuminating with a blue light excitation filter (488 nm) and a green light emission/barrier filter (540-550 nm), lysosomes appear yellowish green instead of red. As this filter combination is very close to the maximum emission of AO, the slide may appear too bright. Excess AO may be removed by washing cells prior to viewing.

AO can be utilized in conjunction with a number of other staining techniques and fluorogenic substrates including ICT's Magic Red®-DEVDase substrate (catalog #936) that detects caspase-3/7 activation in apoptotic cells[8]. Because of the overlap in emissions, be wary of dual staining with other red stains as this will yield confusing results. Red dyes should be used separately.

AO may be used neat or diluted in diH_2O, PBS, or media prior to pipetting into the cell suspension. Lysosomal structures can be visualized by staining with AO at 0.5-5.0 µM. This concentration range can be obtained by diluting ICT's AO reagent stock (catalog #6130 at 1 mM) 1:2,000-1:200 (0.05-0.5% v/v) into the final cell suspension. For example, if using AO at 1.0 µM in the final cell suspension, it must be diluted 1:1,000. First dilute it 1:100 in PBS; e.g., put 10 µL AO into 990 µL PBS. Pipette the diluted AO into the cell suspension at approximately 1:10; e.g., put 50 µL diluted AO into 450 µL cell suspension. Always protect AO from bright light.

SPECIFICATIONS

- 0.5 mL at 1 mM (266 µg/mL)
- CAS number 65-61-2
- Chemical name 3,6-acridinediamine, N, N, N', N'-tetramethylmonohydrochloride
- Molecular formula $C_{17}H_{20}ClN_3$
- Molecular weight 301.82
- Pale red/orange liquid
- pH 5.0 ± 0.5

FLUORESCENCE

- Monomeric form (0.01M phosphate buffer - 0.15M NaCl pH 7.0)[1, 3, 4]:
  Excitation 492 nm
  Emission 525 nm
- Aggregated or DNA complexed form[1, 3, 4]:
  Excitation 502 nm
  Emission 520-524 nm
- Aggregated or RNA complexed form[1, 3, 4]:
  Excitation 457 nm
  Emission 630-644 nm

STORAGE

- 2-8°C.
- Shelf-life up to 24 months when refrigerated and protected from light.

SAFETY

- Acridine orange may be a potent mutagen at high concentrations and probable carcinogen; knowledge about health hazards is incomplete.
- See Safety Data Sheet (SDS) for any warnings. SDS available at www.immunochemistry.com and by calling ICT at 952-888-8788 and 800-829-3194.

HOW TO USE

1. AO may be used neat or diluted in diH_2O, PBS, or media. ICT's catalog #6130 is supplied as 0.5 mL liquid at 1 mM (266 µg/mL).
2. Add AO to the cell sample media at 0.5 - 5 µM, equal to a final dilution of 1:2,000 - 1:200 in the cells (0.05-0.5% v/v). For example, if using AO at 1.0 µM in the final cell suspension, it must be diluted 1:1,000:
   2a. First dilute it 1:100 in PBS or diH_2O; e.g., put 10 µL AO into 990 µL PBS or diH_2O.
   2b. Pipette the diluted AO into the cell suspension at approximately 1:10; e.g., put 50 µL diluted AO into 450 µL cell suspension.
3. Incubate 15-30 minutes at 37°C.
4. Wash cells if reagent is too bright.
5. Analyze with fluorescence:
   5a. Lysosomes will appear yellowish green by illuminating cells with a blue light (488 nm) excitation filter and a green light (540-550 nm) emission/barrier filter (Figures 1A and 2A).
   5b. Alternatively, lysosomes will appear red when using an excitation filter of 550 nm (540-560 nm) and a long pass >610 nm emission/barrier filter (Figure 2B).

REFERENCES

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FIGURE 1: ACRIDINE ORANGE STAINING OF JURKAT CELLS

Normal Jurkat cells stained with Acridine Orange (AO) show orange lysosomal staining. Jurkat cells were stained with 5 µM AO in PBS for 60 minutes at 37°C. Photomicrographs were taken using a Nikon Eclipse E800 photomicroscope using a 460-500 nm excitation filter and a 505-560 nm emission / barrier filter set at 300X. Photo B shows the corresponding DIC image of the cells in A (AO appears faintly). Data courtesy of Dr. Zbigniew Darzynkiewicz (Brander Cancer Research Institute, New York, NY).

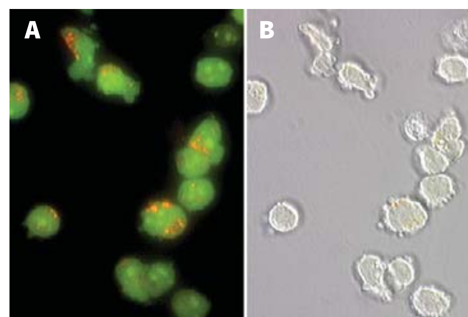
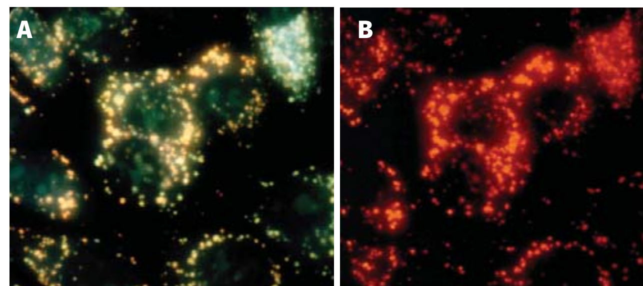


FIGURE 2: ACRIDINE ORANGE STAINING OF MCF-7 CELLS

Apoptosis was induced in human breast cancer MCF-7 cells by treating them with 0.15 µM camptothecin (catalog #6210) for 24 hours at 37°C. Cells were stained with AO in PBS for 30 minutes, then washed twice in PBS. Cells were photographed with a Nikon Microphot-FXA epifluorescence microscope at 40X using either a blue light excitation (492 nm) with a 540-550 nm emission filter (A, lysosomes appear yellowish green), or green light excitation (540 nm) with a long pass >640 nm barrier filter (B, lysosomes appear red). Experiment performed in the laboratory of Dr. Zbigniew Darzynkiewicz (Brander Cancer Research Center Institute, New York, NY)[8].



Thank you for using Acridine Orange!
If you have any questions, or would like to share your data, please contact us at help@immunochemistry.com.

BRIGHT MINDS, BRIGHT SOLUTIONS.
ImmunoChemistry Technologies, LLC gratefully acknowledges the significant contributions made by one of its founders, Brian W. Lee, Ph.D in the development of this product, including the creation and illustration of its strategy and protocol.

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